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Frequency distribution of helminths of wolves in Kazakhstan

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Abstract: Between 2001 and 2008 a total of 41 wolves (*Canis lupus*) were necropsied in southern Kazakhstan and their intestinal parasite fauna evaluated. Of these animals 8 (19.5%) were infected with *Echinococcus granulosus*, 15 (36%) with *Taenia* spp, 13 (31.7%) with *Dypilidium caninum*, 5 (12.2%) with *Mesocostoides lineatus*, 15 (36.6%) with *Toxocara canis*, 16 (39%) with *Toxascaris leonina*, 8 (19.5%) with *Trichuris vulpis*, 9 (22%) with *Macracanthorhynchus catulinus* and 1 (2.4%) with *Moniliformis moniliformis*. All parasites had an aggregated distribution which followed a zero inflated or hurdle model. Although a small convenience sample of wolves, the results indicate a high prevalence of infection with *E. granulosus*. The mean abundance (1275 *E. granulosus* per wolf) was high with individual infected wolves carrying intensities of several thousand parasites. As wolves are common in Kazakhstan they may act as an important host in the transmission of this zoonotic parasite. The wolves were sampled from an area of Kazakhstan where there is a high prevalence of hydatid cysts in livestock and where echinococcosis has been observed in wild ungulates.

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Frequency Distributions of helminths of wolves in Kazakhstan.

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Abstract

Between 2001 and 2008 a total of 41 wolves (*Canis lupus*) were necropsied in southern Kazakhstan and their intestinal parasite fauna evaluated. Of these animals 8 were infected with *Echinococcus granulosus* (19.5%), 15 (36%) with *Taenia* spp, 13 (31.7%) with *Dypilidium caninum*, 5 (12.2%) with *Mesocestoides lineatus*, 15 (36.6%) with *Toxocara canis*, 16 (39%) with *Toxascaris leonina*, 8 (19.5%) with *Trichuris vulpis*, 9 (22%) with *Macracanthorhynchus catulinus* and 1 (2.4%) with *Moniliformis moniliformis* (2.4%). All parasites had an aggregated distribution which followed a zero inflated or hurdle model. Although a small convenience sample of wolves, the results indicate a high prevalence of infection with *E. granulosus*. The mean abundance (1275 *E. granulosus* per wolf) was high with individual infected wolves carrying intensities of several thousand parasites. As wolves are common in Kazakhstan they may act as an important host in the transmission of this zoonotic parasite.

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Key words: *Echinococcus*, wolves, helminths, epidemiology, zero-inflated, Kazakhstan

28 Since independence from the Soviet Union echinococcosis has been an emerging disease in
29 Kazakhstan (Torgerson et al., 2002; 2006). This has been characterised by a dramatic increase in the
30 numbers of human surgical cases recorded. The prevalence of echinococcosis in livestock in
31 Kazakhstan is high (Torgerson et al., 2003a) and there is also a high prevalence in the rural dog
32 population. Prevalences in shepherd dog populations have been recorded at over 20% (Torgerson et
33 al., 2003b). Little is known concerning the role of wild carnivores in the transmission of
34 *Echinococcus granulosus* in Kazakhstan. However wolves (*Canis lupus*) are known to be important
35 definitive hosts in the northern hemisphere (Craig and Craig, 2005).

36 Control and elimination of echinococcosis is possible and has been achieved in several areas
37 such as New Zealand, Tasmania and Cyprus (Craig and Larrieu, 2006). Successful elimination
38 programmes have usually been achieved when the parasite is maintained by a domestic cycle (such
39 as dogs and sheep) and the distribution is limited geographically, such as on an island, making
40 control of reintroduction feasible. Kazakhstan is a large country bordered by other endemic
41 countries. In addition it has one of the world's largest wolf populations which is estimated at
42 between 35,000 and 45,000 individuals (Lobachev and Bekenov, 2003). This provides the
43 opportunity for *E. granulosus* to be maintained in a large susceptible wild life reservoir which could
44 hamper efforts at control of this parasite. Furthermore, little is known with regard to the parasite
45 fauna of wolves and factors such as frequency distribution of parasites that may affect transmission.

46 Because of their high populations, wolves are considered a threat to livestock in many areas of
47 Kazakhstan and there are government incentives to control wolf populations. This study describes
48 the helminthological findings from 41 wolves, shot by hunters in southern Kazakhstan between
49 2001 and 2008. The wolves were from South Kazakhstan, Taras and Almaty Oblasts and shot
50 during the winter months. The gastrointestinal tracts of wolf carcasses supplied by hunters were
51 removed for further examination.

52 Helminths were recovered from the gastrointestinal tract by the method of Skryabin and
53 Petrov (1964). The small intestine was opened along the entire length, the mucosa scraped and the
54 contents flushed with water. The flushing volume was adjusted with water to 2 liters and thoroughly
55 mixed. This was examined in aliquots of 50 ml in Petri dishes using binocular microscope. Cestode
56 species were mounted and stained with carmine, and nematodes cleared in lactic acid with glycerol.
57 Helminth species were morphologically identified according to Abuladze et al. (1990) and Skryabin
58 and Petrov (1964).

59 The data were entered onto an excel spreadsheet and imported into R (www.r-project.org).
60 Summary prevalence for each parasite was calculated and the 95% exact binomial confidence
61 intervals. For analysis of abundance, the best fitting frequency distribution was used a number of

general linear models (GLM) or zero-inflated generalised Poisson models (ZIGP) were used. The optimal frequency distribution for each helminth species was determined as described previously (Ruegg et al., 2008; Ziadinov et al., 2010). The 95% confidence intervals of the mean parasite abundance were calculated from the likelihood profile.

The prevalence, abundance and optimal frequency distributions of each parasite species is given in table 1.

Table 1. Prevalence, abundance and frequency distributions of parasites of wolves in Kazakhstan

Parasite	Prevalence (%) (95% CIs)	Mean Abundance (95% CIs)	Frequency ¹ distribution	Parameters ²
<i>E. granulosus</i>	19.5 (8.8-34.9)	1275 (405-2342)	Zero-inflated negative binomial	0.80(zero) 6532(mean) 2.56 (theta)
<i>Taenia</i> spp	36.6 (22.1-53.1)	1.15 (0.63-1.76)	Zero-inflated Poisson	0.614 (zero) 2.97(mean)
<i>Dipylidium caninum</i>	31.7 (18.1-48.1)	3.39 (1.66-5.46)	Zero-inflated negative binomial	0.675(zero) 8.61 (mean) 2.46(theta)
<i>Mesocestoides lineatus</i>	12.2 (4.1-26.2)	0.82 (0.194-1.804)	Zero-inflated Poisson	0.878(zero) 6.99 (mean)
<i>Toxocara canis</i>	36.6 (22.1-53.1)	5.52 (2.51-9.81)	Zero-inflated negative binomial	0.623(zero) 15(mean) 1.65(theta)
<i>Toxascaris leonina</i>	39 (24.2-55.5)	4.9 (2.39-8.20)	Zero-inflated negative binomial	0.603(zero) 12.54(mean) 2.15(theta)
<i>Trichuris vulpis</i>	19.5 (8.8-34.9)	1.12 (0.46-1.95)	Zero-inflated Poisson	0.80 (zero) 5.86(mean)
<i>Macracanthorhynchus catulinus</i>	22 (10.6-37.6)	1.32 (0.634-2.00)	Zero-inflated Poisson	0.78 (zero) 6.10 (mean)
<i>Moniliformis moniliformis</i>	2.4 (0.06-12.9)	0.19		NA ³

¹ Zero inflated and hurdle models were mathematically indistinguishable with this data.

² The parameters are proportion zero inflated (zero), mean of positive counts (mean) and constant of aggregation (theta for negative binomial only)

³ Only one animal was infected with this parasite, so no meaningful analysis could be undertaken

The best fit model in each case was the hurdle and zero inflated model. *E. granulosus*, *Dipylidium caninum*, *Toxocara canis* and *Toxascaris leonina* had a zero inflated /hurdle negative

78 binomial model with other parasites having a better fit to the zero-inflated/hurdle Poisson model.
79 Hurdle and zero-inflated models were indistinguishable mathematically.

80 This manuscript reports the frequency distribution and prevalence of a number of parasites of
81 wolves in Kazakhstan. A few caveats must be taken when interpreting the data. The data was
82 collected over a number of years and from a large geographical area in southern Kazakhstan. Thus
83 it can not be seen as a random sample, but a convenience sample from materials provided by
84 hunters. However, with such a top level wild predator it would not be appropriate to take a large
85 random sample for specifically a parasitological investigation, even though the numbers of wolves
86 are relatively high in Kazakhstan. Therefore the only realistic method of obtaining such data is from
87 the methodology described.

88 Despite this caveat there are a number of important observations in this study. *E. granulosus*
89 is a common parasite of wolves with 8 of the 41 (19.5%) wolves infected. The abundance of
90 infection was high with an average of 1275 parasites per wolf, or an intensity of 6533 parasites per
91 infected wolf. The prevalence of infection is comparable to that seen in shepherd dogs sampled in
92 the same geographical region. Shepherd dogs had a prevalence of 23% with an abundance of
93 infection of 631 parasites per dog (Torgerson et al., 2003b). However, the earlier study in dogs may
94 underestimate both the prevalence and abundance of infection as this was estimated by arecoline
95 purgation which has poor diagnostic sensitivity (Ziadinov et al., 2008). Thus it appears that wolves
96 are an important host of this zoonotic parasite in Kazakhstan. The source of infection for these
97 wolves is presently unknown, but future genetic analysis of parasite specimens isolated from
98 wolves, livestock and dogs in southern Kazakhstan may give important clues. Livestock in southern
99 Kazakhstan have a high prevalence of cystic echinococcosis (Torgerson et al., 2003a) and thus
100 could provide a source of infection for wolves. Hydatid cysts have also been recorded in wild
101 ungulates in Kazakhstan. These include the saiga antelope, the Siberian roe deer and wild boar
102 (Baytursinov, 2008, 2009). The source of infection for wolves is an important open question. A self
103 sustaining cycle confined entirely to wild life hosts might hamper efforts at control of this parasite
104 especially if the genotypes involved are the same ones that infected domestic livestock and
105 domestic dogs.

106 The abundance and prevalence recorded in the present sample of wolves was lower than that
107 recorded in the USA (Foreyt et al., 2009) where approximately 63% of timber wolves were infected
108 but higher than the 2.9% recorded in Lithuania (Bagraade et al., 2009). The genotypes infecting
109 wolves in Kazakhstan is presently unknown, but in Spain four of 27 Iberian wolves were infected
110 with the G1 strain of *Echinococcus* indicating that wolves may help to maintain the sheep – dog life
111 cycle (Sobrino et al., 2006). Likewise in Bulgaria, wolves were infected with the G1 strain (Breyer

et al., 2004). In the Baltic region the G10 strain may infect wolves (Moks et al., 2006) which is likely to be from wild ungulates.

The frequency distributions of all the parasite species were highly over dispersed. Each parasite had a best fit to a zero inflated or hurdle model. Zero-inflated and hurdle models have been infrequently used in epidemiological studies of animal parasites. However, the computing software to enable this is now available and important ecological information can be gained from such analysis. Previous examples of the use of such models include Denwood et al. (2008) and Ziadinov et al. (2010). In a zero inflation model there could be true or false zeros (Martin et al., 2005). The true zeros in the present data set may be when the parasite is absent from the host. False zeros might arise where the parasite is present but not detected. The sensitivity of necropsy used in the present study is high thus the latter possibility is improbable. For each parasite examined, the model fit was better for a zero inflated or hurdle model than a standard Poisson or negative binomial GLM. A hurdle model assumes that all zeros are true zeros and the non zero count data is modeled as a truncated count distribution. In our data set, the two models are indistinguishable both in terms of parameters and in terms of likelihood. This is because the probability of a zero count given the mean is very low for each parasite in the non-zero inflated proportion of individuals. Our data therefore indicates for each parasite species an animal has been exposed to a variable number of infectious stages and become infected or alternatively has not been exposed. *E. granulosus* metacestodes can contain very large numbers of protoscolices each one capable of developing into an adult tapeworm. Thus a single exposure could result in a large but highly variable number of parasites which can be described by the non zero terms of the relevant negative binomial distribution. With some other parasites such as *Taenia* spp, the infectious insult will be much smaller as with most *Taenia* species a single metacestode has the potential to develop into a single tapeworm. Thus the infected wolves might be more likely to have a distribution consistent with the non zero terms of the Poisson distribution with *Taenia* spp...

In conclusion this study demonstrates that wolves in Kazakhstan have a high prevalence and abundance of infection with *E. granulosus*. It is presently unknown if these wolves are maintaining an entirely wild life cycle becoming infected from prey species or if is spilling over from the domestic cycle through predation of domestic ruminants or scavenging carcasses.

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